Dataset: Results from shipboard high-pressure incubations of diffuse flow vent fluids collected from the Crab Spa and Alvinella sites at East Pacific Rise during the AT26-10 expedition, Jan. 2014 (Microbial Communities at Deep-Sea Vents project)

Project(s): An Integrated Study of Energy Metabolism, Carbon Fixation, and Colonization Mechanisms in Chemosynthetic Microbial Communities at Deep-Sea Vents (Microbial Communities at Deep-Sea Vents)

Abstract: This dataset includes results from shipboard high-pressure incubations of diffuse flow vent fluids collected from the Crab Spa (9.8398º N, 104.2913º W) and Alvinella (9.8398º N, 104.2915º W) sites at East Pacific Rise during the AT26-10 oceanographic expedition in January 2014. Reported parameters include dates and time elapsed, flow rate, temperature, pressure, and pH, and concentrations of NO3, NH4, H2, H2S, CH4. For a complete list of measurements, refer to the supplemental document 'Field_names.pdf', and a full dataset description is included in the supplemental file 'Dataset_description.pdf'. The most current version of this dataset is available at: http://www.bco-dmo.org/dataset/628993

Description: Results of on-board incubations of microbes in diffuse flow vent fluids collected from Crab Spa and Alvinella patch

This dataset includes results from shipboard high-pressure incubations of diffuse flow vent fluids collected from the Crab Spa (9.8398º N, 104.2913º W) and Alvinella (9.8398º N, 104.2915º W) sites at East Pacific Rise during the AT26-10 oceanographic expedition in January 2014. Reported parameters include dates and time elapsed, flow rate, temperature, pressure, and pH, and concentrations of NO3, NH4, H2, H2S, CH4.

Vent fluids used in shipboard incubations were corrected from diffuse flow vent sites at the East Pacific Rise (2503 m): Crab Spa (9.8398º N, 104.2913º W) and Alvinella (9.8398º N, 104.2915º W) (see description in McNichol et al. [2016]). Fluids were collected using isobaric gas-tight samplers [Seewald et al., 2002] prior to their transfer to the shipboard continuous culture system [Foustoukos and Perez-Rodriguez, 2015]. Here, high-pressure incubations (250 bars) were conducted at mesophilic (30 ºC) and thermophilic (50 ºC) conditions to constrain the function and metabolic rates of denitrifying and DNRA microbial communities residing at Crab Spa and Alvinella, respectively. To enhance the activity of nitrate-respiring anaerobic bacteria, an NO3- (5 mm) and H2(aq) (1.30 mM)-enriched medium was introduced in the high-pressure incubations under strictly anaerobic conditions. Dissolved HCO3- (7.3 mm, 13C labeled) was used as added carbon source. Vent fluids were introduced at a flow rate of 0.042 mL/min, while growth medium was added at a rate of 0.0042 mL/min. The two sets of experiments were performed for 356 (Crab Spa) and 50 hours (Alvinella). Direct cell counts were conducted by staining cells with 0.1% acridine orange and counting them with a fluorescence microscope. 15N/14N isotopic analysis of the NO3-, NH4+ and biomass were conducted with a Thermo Scientific Delta VPlus mass spectrometer and CE Instruments NA 2500 series elemental analyzer (EA).

References:

Foustoukos, D., and I. Perez-Rodriguez (2015), A continuous culture system for assessing microbial activities in the piezosphere, Applied and Environmental Microbiology, 81(19), 6850-6856.

McNichol, J., S. P. Sylva, F. Thomas, C. D. Taylor, S. M. Sievert, and J. S. Seewald (2016), Assessing microbial processes in deep-sea hydrothermal systems by incubation at in situ temperature and pressure, Deep Sea Research Part I: Oceanographic Research Papers, 115, 221-232.

Seewald, J. S., K. W. Doherty, T. R. Hammar, and S. P. Liberatore (2002), A new gas-tight isobaric sampler for hydrothermal fluids, Deep-Sea Research, Part I: Oceanographic Research Papers, 49(1), 189-196.

Acquisition Description:

From AT26-10 cruise report (01/29/2014):

DOB: An Integrated Study of Energy Metabolism, Carbon Fixation, and Colonization Mechanisms in Chemosynthetic Microbial Communities at Deep-Sea Vents

Cruise Report by the CIW research team: Dr. Ileana Perez-Rodriguez, Mr. Matt Rawls and Dr. Dionysis I. Foustoukos

The CIW team was responsible for the shipboard continuous culturing incubations of vent fluids collected from Crab Spa and Tica hot springs during the AT26-10 expedition at 9oN EPR by utilizing our high-pressure bioreactor (Fig. 1). This was accomplished through a collaborative effort with Jeff Seewald and Sean Sylva (WHOI), who deployed isobaric gas-tight samplers (IGTs) to collect hydrothermal vent fluids at the diffuse flow sites. Experiments were designed to study the cycling to N through the metabolic processes of denitrification and dissimilatory nitrate reduction to ammonia (DNRA) under in-situ deep-sea vent temperature and pressure conditions.

We studied the evolution of nitrate reducing microorganisms at mesophilic (30oC) and thermophilic (50oC) conditions at pressures ranging from 5 to 250 bar. Vent fluids (16 IGTs) were delivered in the bioreactor and homogeneously mixed with aqueous media solution enriched in dissolved nitrate, hydrogen and 13C labeled bicarbonate to facilitate the growth of nitrate reducing microorganisms (Fig. 2). The two distinct sets of experiments were lasted for 356 and 100 hours. In short, experimental results constrained the function and metabolic rates of the denitrifying microbial communities in the Crab Spa fluids, while DNRA metabolic pathways were identified for the populations residing in the moderate temperature vent fluids (60oC) of the Alvinella colony at Tica.

During the course of the experiments we monitored the growth of deep-sea microbial communities by measuring the concentrations of dissolved aqueous species directly involved in nitrate based metabolism, such as NO3, NH4, H2 and H2S. We also monitored cell densities by utilizing an epi-fluorescence microscope (Sievert, WHOI). Dissolved gas and NH4+ concentrations were attained by gas and ion chromatography (Seewald - Sylva, WHOI). Subsamples were also collected for a number of offshore analysis to determine: i) the 15N/14N isotope composition of NO3-,/NH4+ and constrain kinetic isotope effects associated with denitrification/DNRA (Perez-Rodriguez, CIW), ii) to study the rates of autotrophic carbon fixation by NanoSIMS (Musat, UFZ), iii) to perform single cell genomics on

the microbial populations grown in the bioreactor (Ramunas, Bigelow) and (iv) to isolate and characterize novel microogranisms from the communities cultured in our experiments (Perez-Rodriguez, CIW and Vetriani, Rutgers).

Processing Description:

BCO-DMO Processing:

- added conventional header with dataset name, PI name, version date
- renamed parameters to BCO-DMO standard
- concatenated the 2 datasets: Crab Spa and Alvinella patch
- blank cells replaced with 'nd'
- added columns for description, date_start and date_end
- version 2017-02-07 replaced version 2015-12-17: added cell concentration, d15N_NO3_ppt, and d15N_Biomass_ppt

Deployment Information

Deployment description for R/V Atlantis AT26-10

Samples were collected by ROV Jason II at the 9N deep-sea hydrothermal vent field on the East Pacific Rise, Pacific Ocean

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Instrument Information

Instrument	
Description	Olympus BX61 microscope with a UPlanF1 100x (numerical aperture, 1.3) oil immersion objective
Generic Instrument Name	Microscope-Optical
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

Instrument	
Description	JSM-6500F field emission scanning electron microscope (JEOL)
Generic Instrument Name	Microscope-Electron
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of electrons behaving as waves.

Instrument	IGT Sampler
Description	*local description not specified*
Generic Instrument Name	Isobaric Gas-Tight Sampler
Generic Instrument Description	Isobaric Gas Tight (IGT) samplers, designed and built by scientists and engineers at WHOI, are titanium instruments designed to be used with deep submergence vehicles to sample corrosive hydrothermal vent fluids at high temperature and high pressure. The IGT prevents the sampled fluid from degassing as pressure decreases during the vehicle's ascent to the surface.

Instrument	custom high pressure bioreactor
Description	The integrated system allows for the culturing of microorganisms under hydrostatic pressures from 0.1 to 69 MPa (and up to 138 MPa with ongoing developments) and at temperatures ranging from 25 to 120°C. For full description, see Foustoukos and Perez-Rodriguez (2015), Applied and Environmental Microbiology, 81, 6850
Generic Instrument Name	Shipboard Incubator
Generic Instrument Description	A device mounted on a ship that holds water samples under conditions of controlled temperature or controlled temperature and illumination.